Environmental Health Perspectives
Vol. 88, pp. 49–55, 1990

Rat and Human Liver Cytosolic Epoxide Hydrolases: Evidence for Multiple Forms at the Level of Protein and mRNA

by Helmut Thomas,* Ludwig Schladt,* Johannes Doehmer,* Michael Knehr,* and Franz Oesch*

Two forms of human liver cytosolic epoxide hydrolase (cEH) with diagnostic substrate specificity for *trans*-stilbene oxide (cEH_{TSO}) and *cis*-stilbene oxide (cEH_{CSO}) have been identified, and cEH_{CSO} was purified to apparent homogeneity. The enzyme had a monomer molecular weight of 49 kDa and an isoelectric point of 9.2. Pure cEH_{CSO} hydrolyzed CSO at a rate of 145 nmole/min/mg. TSO was not metabolized at a detectable level, and like cEH_{TSO}, the enzyme was about three times more active at pH 7.4 than at pH 9.0. Unlike cEH_{TSO}, cEH_{CSO} was efficiently inhibited by 1 mM 1-trichloropropene oxide (90.5%) and 1 mM STO (92%). Similarly, liver cEH purified 541-fold from fenofibrate induced Fischer 344 rats was shown to be a native 120 kDa dimer of two 61 kDa subunits. The enzyme expressed maximum activity of 205 nmole/min/mg at pH 7.4 toward the diagnostic substrate TSO with an apparent K_m of 1.7 μM. In Western blots, polyclonal antibodies against rat liver cEH were shown to recognize a single 61 kDa protein band from liver cytosol of rat, mouse, guinea pig, Syrian hamster, and rabbit. This antibody precipitated neither human liver cEH_{TSO} or cEH_{CSO}. Antibodies against rat liver microsomal epoxide hydrolase reacted with cEH_{CSO} in the Western blot and on immunoprecipitation. Using antibodies against rat liver cEH, 24 positive clones were picked upon colony blot screening of a pEX 1/*E. coli* POP 2136 expression library. After Northern blotting against rat liver total RNA, clone 24 recognized a second mRNA-species besides the putative message for cEH_{TSO}, thus providing strong evidence for the multiplicity of rat liver cEH as well. The importance of these findings for the control of reactive metabolites of various origins is discussed.

Introduction

Epoxides are formed *in vivo* mainly by the microsomal cytochrome P-450-dependent monooxygenase system. Epoxides have been identified as intermediates of arachidonic acid (*1*) and steroid (*2*) metabolism as well as products of fatty acid peroxidation (*3*). Epoxides have also been identified as metabolites of numerous xenobiotic compounds containing olefinic or aromatic double bonds (*4*). Due to their electrophilicity, epoxides may bind covalently to cellular nucleophiles such as proteins and nucleic acids and thus elicit toxic, mutagenic or carcinogenic effects (*5*).

Enzymatic inactivation of reactive epoxides is provided in part by conjugation to glutathione (*6*) or the addition of water by epoxide hydrolases. Epoxide hydrolases (EC 3.3.2.3) constitute a family of enzymes comprising at least three major forms. The major forms have been characterized as distinct proteins on the basis of subcellular localization, molecular weight, optimum pH, substrate specificity, immunological properties, inhibition, and different response to known inducers (*7*). Purified microsomal epoxide hydrolase (mEH_b) has a molecular weight of about 50 kDa and hydrolyzes a wide variety of cyclic epoxides such as phenanthrene-9,10-oxide and benzo(*a*)pyrene-4,5-oxide (*8*) as well as mono- and *cis*-1,2-disubstituted oxiranes (*9*). Microsomal cholesterol epoxide hydrolase (mEH_{Ch}) is distinguished by its narrow substrate specificity for physiologically arising 5,6-epoxy-cholesterol and its derivatives (*10*).

In contrast, epoxides on cyclic systems are poor substrates for cytosolic epoxide hydrolase (cEH), which appears to be localized in peroxisomes as well as in the cytosol (*11*). This enzyme rapidly hydrolyzes aliphatic oxiranes, such as epoxides of *cis*- and *trans*-unsaturated fatty acids, including arachidonic acid, squalene oxides, and side chain epoxidized sterols (*7*). A diagnostic *in vitro* substrate for cEH is *trans*-stilbene oxide (TSO), which is neither metabolized by mEH_b nor mEH_{Ch}. Conversely, benzo(*a*)pyrene-4,5-oxide and styrene oxide (STO) are used as selective substrates for mEH_b.

Most of our knowledge about cEH has been gained from studies on the purified enzymes from mouse and rabbit liver (*12*). These two species, together with the hamster, possess by far the highest TSO-hydrolyzing

*Institute of Toxicology, University of Mainz, D-6500 Mainz, Federal Republic of Germany.

Address reprint requests to H. Thomas, Institute of Toxicology, University of Mainz, D-6500 Mainz, Federal Republic of Germany.

activity of all investigated species (165–335 nmole/min × g liver and 106–129 nmole/min × g liver compared to human and rat liver with 15–22 nmole/min × g and 10–15 nmole/min × g, respectively) (*13*). The low specific cytosolic activity of rat and human liver cEH as well as the strong inducibility of the rat liver enzyme by hypolipidemic drugs with peroxisome proliferating activity suggested that these enzymes differ in properties and regulation and that rat liver cEH may actually be associated with peroxisomes and play a distinct role in the metabolic processes of this subcellular particle.

The different properties of cEH and mEH_b as well as the substantial differences among cEH activities in various species suggest that there are differences in detoxification capacities and protection of subcellular compartments from reactive epoxides. The largely unknown properties of human liver cEH make extrapolation of functional parameters and risk assessment with respect to environmental toxicants difficult. This investigation was therefore directed toward elucidating possible multiplicities of cEH in rats and humans, the properties of these isoenzymes, and their contribution to the cellular protection against reactive epoxides.

Purification of Rat and Human Liver cEH

Cytosolic epoxide hydrolase was purified to apparent homogeneity from fenofibrate-induced rat liver (*14*). Throughout the purification, enzyme activity was monitored with the substrates STO, CSO, TSO, and TESO, and there was no evidence from the elution profiles for activities other than STO, TSO, or TESO hydrolases, which coincided in all chromatograms. Also, the purification factors of 423, 541, and 485 for STO, TSO, and TESO hydrolase activity, respectively, were of the same order of magnitude. Additionally, cEH activity of untreated and induced animals expressed identical elution behavior in the corresponding chromatography steps. Further comparing the specific activity toward TSO of purified cEH from induced animals (173 nmole/min × mg) with 62 nmole/min × mg for the estimated 30% pure enzyme from controls indicated that the two enzyme forms were identical. There was no evidence for a multiplicity of cEH in rat liver at the protein level.

Investigation of human liver cEH from cytosol revealed that CSO, which is regarded a diagnostic substrate for mEH_b, was hydrolyzed at the considerable rate of about 1.3 nmole/min × mg, which is slightly higher than the turnover by monkey (*Macaca fascicularis*) and more than 10 times higher than the hydration of CSO by rat liver cytosol (*13*). Isoelectric focusing confirmed the existence of two distinct human liver cytosolic epoxide hydrolases for the hydration of CSO (cEH_{CSO}) and TSO (cEH_{TSO}), and the specificities of both proteins were mutually exclusive. cEH_{TSO} was very unstable and could only be partially purified by anion exchange chromatography and gel filtration (Fig. 1).

In contrast, cEH_{CSO} was purified 85-fold to apparent homogeneity from 60 g of liver rom a 23-year-old male organ donor who had died in an accident (*15*) (Fig. 2).

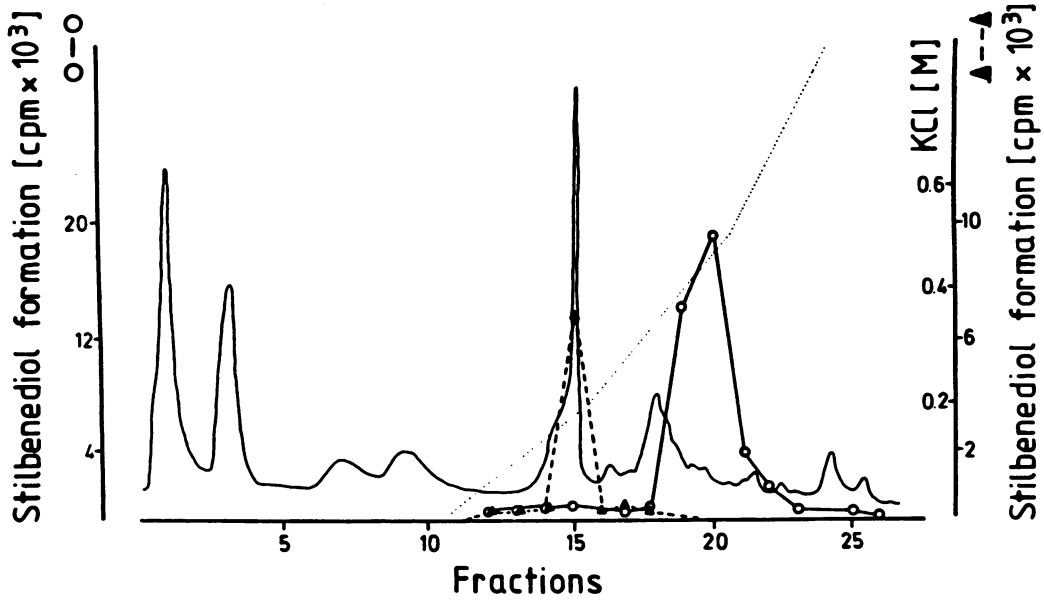

FIGURE 1. Fractionation of human liver cytosolic epoxide hydrolase by anion-exchange chromatography. Cytosolic protein, 8 mg, was fractionated on a Mono Q column (FPLC) at a flow rate of 1 mL/min, and eluates were assayed for epoxide hydrolase activity toward CSO and TSO.

Biochemical Properties of Purified Rat and Human Liver cEH

Native rat liver cEH elutes as a protein of molecular weight 120 kDa during gel filtration on Sephadex G-150 and yields a single band after SDS-PAGE corresponding to a molecular weight of 60 kDa. This suggests a native dimer of two closely related, if not identical, subunits (*14*) (Fig. 2).

Similarly, gel filtration of human liver cytosol on Superose-12 revealed a native molecular weight of approximately 130 kDa for TSO-hydrolyzing activity, which is close to 140 kDa reported by Wang et al. (*16*) for purified human liver cEH_{TESO}. Native cEH_{CSO} eluted essentially with the void volume, indicating the formation of high molecular weight aggregates that are resolved on SDS-PAGE into a single protein band of molecular weight 49 kDa (Fig. 2). This behavior resembles closely that of mEH_b from all species investigated so far (*7*). However, the pI of 9.2 for cEH_{CSO} is different from pI 7.0 for human liver mEH_b and pI 5.7 for cEH_{TSO}. Also, the optimum pH for cEH_{CSO} of 7.4 is far from pH 9.0, which is known as the pH optimum for mEH_b, and it is identical with the pH optimum of 7.4 for rat liver cEH (*14*) and human liver cEH_{TSO}. A relationship of cEH_{CSO} to the classical cytosolic epoxide hydrolases may be deduced from a similar response of cEH_{CSO} to the inhibitors benzil, 1-benzylimidazole, and chalcone, whereas inhibition of cEH_{CSO} by TCPO or STO would rather argue for a mEH_b-like isoenzyme (Table 1).

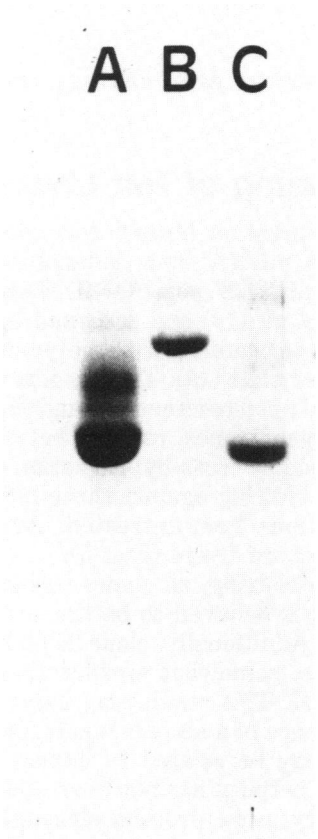

FIGURE 2. SDS-polyacrylamide gel electrophoresis of purified human liver cEH_{CSO}. Rat liver mEH_b, 7μg (lane A) 1 μg of rat liver cEH (lane B), and 1.5 μg purified human liver cEH_{CSO} (lane C) were electrophoresed in a 12% slab gel according to Laemmli (*21*) and stained with Coomassie brilliant blue R-250.

Table 1. Effect of different modulators on the activity of human liver cytosolic epoxide hydrolase.

	Epoxide hydrolase activity, % control			
	Microsomal with			
Modulator	STO	CSO	cEH_{CSO} with CSO[a]	cEH_{TSO} with TSO[a]
TCPO	2	5	9.5	69
Benzil	219	49	74	68
1-Benzylimidazole	224	38	91	105
Chalcone	107	48	60	52
STO	54	1	8	90

[a] Activities of cEH_{CSO} and cEH_{TSO} were determined after separation by anion exchange chromatography as described by Schladt et al. (*15*). Incubations contained the individual compounds at a concentration of 1 mM.

Unlike purified mouse liver cEH, which readily hydrates CSO at a rate of 136 nmole/min × mg, approximately one-seventh the rate of TSO-hydrolysis (*17*), rat liver cEH does not metabolize CSO at a detectable level and hydrates TSO at a rate of 173 nmole/min × mg in the presence of 26 μM substrate. The corresponding apparent K_m and V_{max} values were determined from Lineweaver-Burk plots as 1.7 μM and 205 nmole/min × mg, respectively.

Purified human liver cEH_{CSO}, on the other hand, converts CSO at a rate of 145 nmole/min × mg, like mouse liver cEH, but does not accept TSO as a substrate. Partially purified cEH_{TSO} does not metabolize STO, in contrast to purified mouse (*17*) and rat liver cEH as well as human liver cEH_{TESO} (*16*) and cEH_{CSO}.

Immunological Properties of Rat and Human Liver cEH

With polyclonal antibodies against purified rat liver cEH, a single band was obtained after Western blotting of purified rat liver cEH and cytosol from control as well as clofibrate-induced animals. Strong induction of this enzyme by the hypolipidemic compound was clearly demonstrated (Fig. 3*A*). The antibody also recognized proteins with a molecular weight of about 60 kDa (corresponding to rat liver cEH) from liver cytosols of mouse, Syrian hamster, and New Zealand white rabbit (Figs. 4*C*,*E*,*F*). The reaction with guinea pig liver cytosol (Fig. 4*D*) was very weak, and there was no cross-reactivity with liver cytosol from green monkey (Fig. 4*G*). The two low molecular weight proteins in Syrian hamster cytosol that were distinguished by the antibody may be products of cEH-proteolysis.

Immunoprecipitation with antiserum against rat liver cEH demonstrated essentially 100% removal from rat liver and kidney cytosol of TSO hydrolyzing activity,

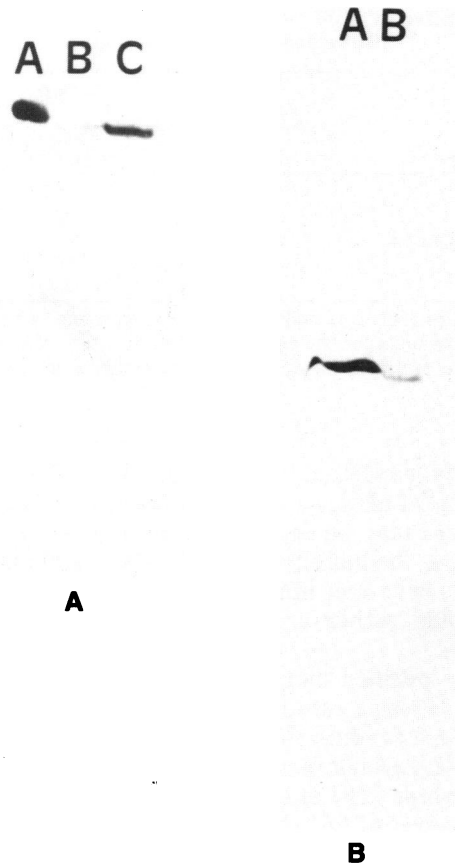

FIGURE 3. Western blotting of rat (*A*) and human liver (*B*) epoxide hydrolases. (*A*) Purified rat liver cEH, 0.16 μg (lane A); 100 μg each of liver cytosol from untreated rats (lane B) and clofibrate-induced rats (0.25% for 1 week in the diet) (lane C) was transferred to nitrocellulose following SDS-PAGE and stained with rabbit anti-rat liver cEH antibodies as described by Towbin (*22*). (*B*) Immunoblotting of 0.9 μg purified rat liver mEH_b (lane A) and 1.5 μg cEH_{CSO} with anti-rat liver mEH_b antibodies (lane B).

whereas human liver cEH_{TSO} was not precipitated at all, and precipitation of cEH_{CSO} did not exceed 20% of the initial activity even at the highest antiserum concentration (*14,15*). However, essentially all human liver cytosolic CSO hydrolase as well as STO hydrolase activity was precipitated with a polyclonal antibody against purified rat liver mEH_b, while the TSO-hydrolyzing activity remained unaffected.

Although the immunological relationship of rat liver mEH_b and cEH_{CSO} was confirmed by Western blotting, the small degree of similarity was underlined by the weak response of about one-tenth the signal intensity at 7.7 times the protein concentration (Fig. 3*B*).

From these immunological investigations one can conclude that rat, mouse, hamster, and rabbit liver as well as rat kidney TSO-hydrolase activities reside on structurally related proteins. On the other hand, human liver cEH_{TSO} and cEH_{CSO} as well as green monkey and guinea pig liver cytosolic TSO-hydrolase appear to be structurally completely different from rat liver cEH. A distant, but definite structural relationship of rat liver

FIGURE 4. Western blotting of cytosolic epoxide hydrolases from different species. Purified rat liver cEH, 0.16 μg (lane A) and 100 μg each of liver cytosol from control rat (lane B), mouse (lane C) guinea pig (lane D), Syrian hamster (lane E), rabbit (lane F), and green monkey (lane G) was transferred to nitrocellulose after SDS-PAGE and stained with rabbit anti-rat liver cEH antibodies.

mEH_b with human liver cEH_{CSO} could be demonstrated.

cDNA Cloning of Rat Liver cEH

A cDNA expression library was constructed using purified poly-A mRNA from tiadenol-induced rat liver in the plasmid pEX1/*E. coli* POP 2136 system according to Haymerle et al. (*18*) and screened by a colony blot hybridization procedure with polyclonal antibodies against rat liver cEH (*19*). Twenty-four positive clones were confirmed upon rescreening, and the cDNA inserts of the four biggest clones, ranging between 0.8 and 1.2 kb, were verified by cross-hybridization using Southern and Northern blotting against three independent total RNA preparations from untreated, Aroclor 1254- and tiadenol-induced rat livers (Fig. 5).

In Northern blotting, all clones recognized the same mRNA, which is believed to be the coding mRNA for rat liver cEH. Additionally, clone 24 picked up a second mRNA species, somewhat smaller than the putative mRNA for cEH. This result may indicate for the first time the existence of a second isoenzyme of cEH in rat liver, which may be related to human liver cEH_{CSO}. However, this activity has not been allocated as yet to a distinct rat cytosolic protein, although minute CSO-hydrolyzing activities of about 0.1 nmole/min × g have been described in rat liver (*13*). Recently, a cytosolic hepoxilin epoxide hydrolase with molecular weight 53 kDa and marginal activity toward STO was isolated from rat liver (*20*) and may correspond to the newly identified mRNA.

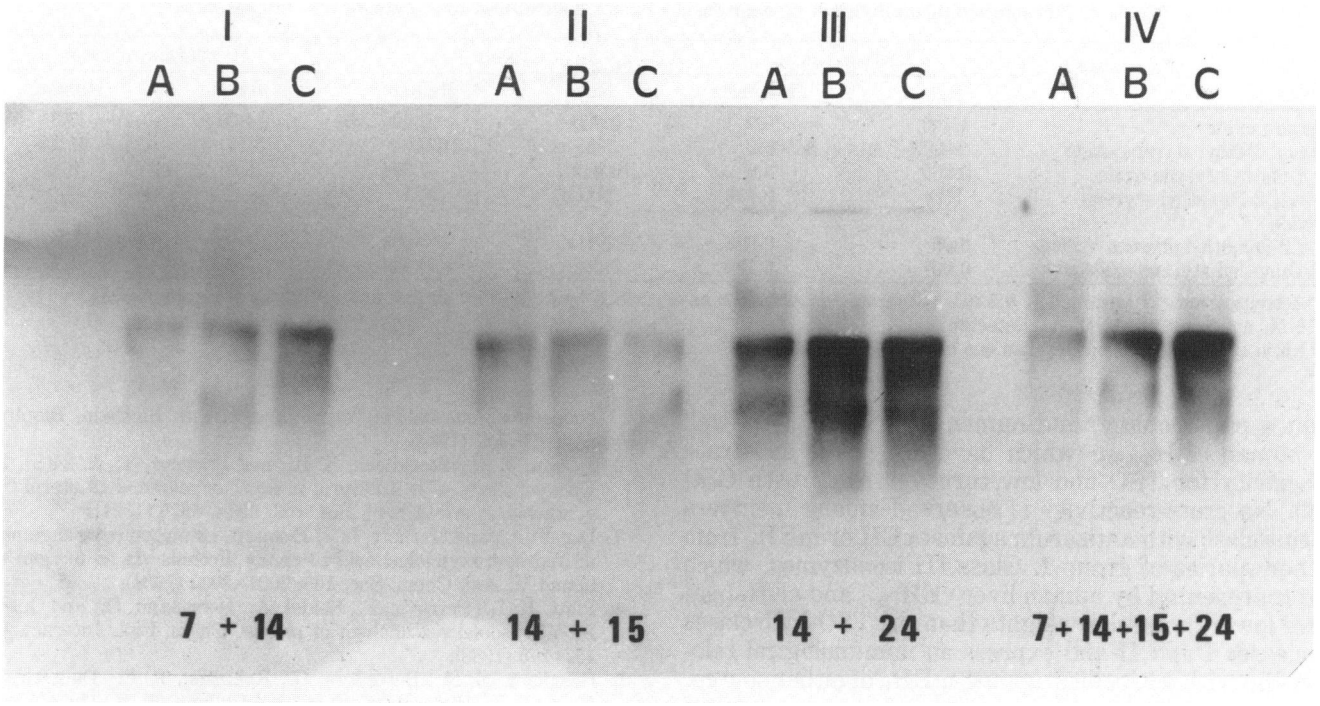

FIGURE 5. Hybridization of cDNA inserts from positive cEH clones with total rat liver RNA. Total RNA from control (lane A), Aroclor 1254-induced (lane B), and Tiadenol-induced (lane C) rat liver was subjected in four identical sets (I–IV) to denaturing 0.7% agarose gel electrophoresis, transferred to nitrocellulose sheets and hybridized with [^{32}P]-labeled cDNA probes from clones 7 + 14 (I); 14 + 15 (II); 14 + 24 (III); and 7, 14, 15, 24 (IV). Hybridization was visualized by autoradiography.

Table 2. Classification of cytosolic epoxide hydrolases from different species according to physical, biochemical, and immunological properties.

		Activity toward[a]					Cross-reactivity with antiserum against[b]		
Class	Species	TSO	CSO	STO	MW	pI	cEH	mEH_b	Reference
I	Mouse liver	939–1500	136–180	475–622	59000	5.5	+	–	(*17,23,24*)
	Rat liver	173	ND[c]	1567	61000	5.5	+	–	(*14*)
	Rabbit liver	0.69–0.93[d]	NM	1380	57000	6.0	+	–	(*25*)[e]
	Hamster liver	2.10–2.20[d]	0.11–0.15[d]	NM	57000	NM	+	–	(*13*)[e]
II	Guinea pig liver	0.87–0.99[d]	0.15–0.21[d]	NM	57000	7.4	–	–	(*13*)[e]
	Monkey liver	0.36–0.44[d]	0.94–0.96[d]	NM	58000	NM	–	–	(*13*)[e]
	Human liver cEH_{TSO}	1.02[f]	0.32	ND	NM	5.7	–	–	(*15*)
	Human liver cEH_{TESO}	150	NM	22	58000	5.1–6.1	–	–	(*15,16*)
III	Human liver cEH_{CSO}	ND	145	ND	49000	9.2	–	+	(*15*)
	Human liver cEH_{PNSO}	14	NM	44	50000	NM	–	+	(*16*)

[a] Activities are given in nmole/min/mg.
[b] (+) Indicates weak but clearly detectable cross-reactivity; (–) indicates very faint reactivity.
[c] ND, not detectable; NM, not measured.
[d] Activities were determined from cytosol.
[e] See also Figure 4.
[f] Activity after anion exchange chromatography of cytosol.

Conclusions

Two forms of human liver cEH, cEH_{CSO}, and cEH_{TSO} have been identified, and cEH_{CSO} as well as rat liver cEH have been purified to apparent homogeneity. An attempt has been made to assign these enzyme forms to three classes (Table 2) according to their physical, biochemical, and immunological properties, taking into account the known properties of cEH from other species.

Class I cEH isoenzymes are characterized by immunological cross-reactivity with either cEH-antiserum against any member of this group and diagnostic substrate specificity toward TSO, STO,and with some limitations, towards CSO. Mouse, rat, rabbit, and hamster liver cEH meet these requirements. Class II comprises

Table 3. Metabolism of methylated styrene oxides by rat and human liver cytosol and microsomes.[a]

	Microsomes		Cytosol		Microsomes/cytosol	
Substrate	Rat	Human	Rat	Human	Rat	Human
Styrene oxide	4.300	40.7	0.300	5.600	14.3	7.3
trans-2-Methyl styrene oxide	NM[b]	86500.0	NM	2100.0	NM	41.2
cis-2-Methylstyrene oxide	0.260	5.4	0.010	0.664	26.0	8.1
trans-1,2-Dimethyl styrene oxide	ND	0.238	ND	ND	—[c]	—
cis-1,2-Dimethyl styrene oxide	0.08	0.665	ND	0.034	—	19.6
2,2-Dimethyl styrene oxide	0.007	0.130	0.001	0.122	7.0	1.1

[a] Activities were determined at 0.5 mM substrate concentration as described by Milbert et al. (*26*) and are given in nmole/min/mg.
[b] NM, not measured; ND, not detectable.
[c] Ratio cannot be formed because one of the values is zero.

guinea pig, monkey, and human liver cEH_{TSO} as well as human cEH_{TESO}, which have diagnostic substrate specificity for TSO and low turnover rates with CSO (*13*). No cross-reactivity is observed among members of this class with antiserum against cEH or mEH_b from either species of group I. Class III isoenzymes, which are represented by human liver cEH_{CSO} and cEH_{PNSO}, have lower molecular weights than the TSO-hydrolases of classes I and II and express an immunological relationship with antibodies against mEH_b of either source.

From this classification it appears that the structural and functional multiplicity of cEH increases with phylogenetic differentiation. The possible impact of this cEH differentiation on the cellular protection against reactive epoxides was investigated with differentially methylated styrene oxides and microsomes or cytosol as enzyme source (Table 3). Human enzymes of either source metabolized all model substrates more efficiently than the corresponding rat liver enzymes. The ratio of microsomal/cytosolic hydration for a certain substrate is clearly shifted toward two to seven times lower values in humans. This finding can be explained by the presence of cEH_{CSO}, which provides mEH_b-like detoxification capacities particularly for *cis*-substituted epoxides such as *cis*-2-methyl styrene oxide, *cis*-1,2-dimethylstyrene oxide, and 2,2-dimethyl styrene oxide.

In conclusion, a multiplicity of human as well as rat liver cEH could be demonstrated at the enzyme level for the human, and at the mRNA level for rat liver cEH. However, due to human liver cEH_{CSO}, this multiplicity appears much more effective in humans than in rats for the protection of different subcellular compartments against reactive epoxides, and human liver seems to be far more efficient in detoxification of reactive epoxides by epoxide hydrolase. Rat liver may therefore not be an appropriate model for epoxide hydrolase-related risk assessment in humans.

The authors thank I. Böhm and H. Steed for typing the manuscript. This investigation was supported by the Deutsche Forschungsgemeinschaft (SFB 302). L. Schladt was the recipient of a postdoctoral fellowship from the "Deutsche Forschungsgemeinschaft," and M. Knehr was supported by a stipendium from the Johannes Gutenberg-Universität Mainz (Titel 68102).

REFERENCES

1. Capdevila, J., Pramanik, B., Napoli, J. L., Manna, S., and Falck, J. R. Arachidonic acid epoxidation: epoxyeicosa-trienoic acids are endogenous constituents of rat liver. Arch. Biochem. Biophys. 231: 511–517 (1984).
2. Nelson, J. A., Steckbeck, S. R., and Spencer, T. A. 24(S), 25-Epoxycholesterol is a natural product of mammalian sterol biosynthesis. J. Am. Chem. Soc. 103: 6974–6975 (1981).
3. Dix, T. A., and Marnett, L. J. Hematin-catalyzed rearrangement of hydroperoxylinoleic acid to epoxy alcohols via an oxygen rebound. J. Am. Chem. Soc. 105: 7001–7002 (1983).
4. Platt, K. L., Petrovic, P., Seidel, A., Beermann, D., and Oesch, F. Microsomal metabolism of picene. Chem.-Biol. Interact. 66: 157–175 (1988).
5. Gozukara, E. M., Belvedere, G., Robinson, R. C., Deutsch, J., Coon, M. J., Guengerich, F. P., and Gelboin, H. V. The effect of epoxide hydratase on benzo(a)pyrene diolepoxide hydrolysis and binding to DNA and mixed-function oxidase proteins. Mol. Pharmacol. 19: 153–161 (1981).
6. Ketterer, B. Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis. Mutat. Res. 202: 343–361 (1988).
7. Wixtrom, R. N., and Hammock, B. D. Membrane-bound and soluble fraction epoxide hydrolases: methodological aspects. In: Biochemical Pharmacology and Toxicology: Methodological Aspects of Drug Metabolizing Enzymes, Vol. I (D. Zakim and D. A. Vessey, Eds.), John Wiley and Sons, New York, 1985, pp. 1–93.
8. Bentley, P., Schmassmann, H., Sims, P., and Oesch, F. Epoxides derived from various polycyclic hydrocarbons as substrates of homogeneous and microsome-bound epoxide hydratase. Eur. J. Biochem. 69: 97–103 (1976).
9. Oesch, F., Kaubisch, N., Jerina, D. M., and Daly, J. W. Hepatic epoxide hydrase. Structure-activity relationships for substrates and inhibitors. Biochemistry 10: 4858–4866 (1971).
10. Sevanian, A., and McLeod, L. L. Catalytic properties and inhibition of hepatic cholesterol-epoxide hydrolase. J. Biol. Chem. 261: 54–59 (1986).
11. Waechter, F., Bentley, P., Bieri, F., Stäubli, W., Völkl, A., and Fahimi, H. D. Epoxide hydrolase activity in isolated peroxisomes of mouse liver. FEBS Lett. 158: 225–228 (1983).
12. Meijer, J., and DePierre, J. W. Cytosolic epoxide hydrolase. Chem.-Biol. Interact. 64: 207–249 (1988).
13. Meijer, J., Lundqvist, G., and DePierre, J. W. Comparison of the sex and subcellular distributions, catalytic and immunochemical reactivities of hepatic epoxide hydrolases in seven mammalian species. Eur. J. Biochem. 167: 269–279 (1987).
14. Schladt, L., Hartmann, R., Wörner, W., Thomas, H., and Oesch, F. Purification and characterization of rat-liver cytosolic epoxide hydrolase. Eur. J. Biochem. 176: 31–37 (1988).
15. Schladt, L., Thomas, H., Hartmann, R., and Oesch, F. Human liver cytosolic epoxide hydrolases. Eur. J. Biochem. 176: 715–723 (1988).
16. Wang, P., Meijer, J., and Guengerich, F. P. Purification of human liver cytosolic epoxide hydrolase and comparison to the microsomal enzyme. Biochemistry 21: 5769–5776 (1982).
17. Meijer, J., and DePierre, J. W. Properties of cytosolic epoxide hydrolase purified from the liver of untreated and clofibrate-treated mice. Characterization of optimal assay conditions, sub-

strate specificity and effects of modulators on the catalytic activity. Eur. J. Biochem. 150: 7–16 (1985).
18. Haymerle, H., Herz, J., Bressan, G. M., Frank, R., and Stanley, K. K. Efficient construction of cDNA libraries in plasmid expression vectors using an adaptor strategy. Nucl. Acids Res. 14: 8615–8624 (1986).
19. Huynh, T. V., Young, R. A., and Davis, R. W. Constructing and screening cDNA libraries in λgt10 and λgt11. In: DNA Cloning, Vol. 1 (D. M. Glover, Ed.), IRL Press, Oxford, 1985.
20. Pace-Asciak, C. R., and Lee, W. -S. Purification of hepoxilin epoxide hydrolase from rat liver. J. Biol. Chem. 264: 9310–9313 (1989).
21. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685 (1970).
22. Towbin, H., Staehelin, T., and Gordon, J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA 76: 4350–4354 (1979).
23. Gill, S. S. Purification of mouse liver cytosolic epoxide hydrolase. Biochem. Biophys. Res. Commun. 112: 763–769 (1983).
24. Gill, S. S. Immunological similarity of epoxide hydrolase activity in the mitochondrial and cytosolic fractions of mouse liver. Biochem. Biophys. Res. Commun. 122: 1434–1440 (1984).
25. Waechter, F., Merdes, M., Bieri, F., Stäubli, W., and Bentley, P. Purification and characterization of a soluble epoxide hydrolase from rabbit liver. Eur. J. Biochem. 125: 457–461 (1982).
26. Milbert, U., Wörner, W., and Oesch, F. Characterization of rat hepatic and renal glutathione S-transferases. In: Primary Changes and Control Factors in Carcinogenesis (T. Friedberg and F. Oesch, Eds.), Deutscher Fachschriften-Verlag, Wiesbaden, FRG, 1986, pp. 14–21.